Journal of the Islamic University of Gaza, (Series of Natural Studies & Engineering) Vol.13, No.1, P.165-173, 2005

PREVALENCE OF MICROORGANISMS ASSOCIATED WITH INTRAMAMMARY INFECTION IN COWS AND SMALL RUMINANTS IN THE NORTH OF PALESTINE

Ghaleb Adwan[1], Dauod Abusafieh[2], Rateb Aref[3] and Jamal Abo Omar[3]

1. Assistant professor, Department of Biology and Biotechnology, An-Najah University

2. Research assistant, Faculty of Agriculture, An-Najah N. University

3. Faculty of Agriculture, An-Najah N. University

Fax: 972-9-2675891 e-mail address: adwang@najah.edu

Running title: subclinical mastitis in Palestine

مدى انتشار الكائنات الحية الدقيقة المصاحبة لالتهاب الضرع دون السريري في الأغنام ، الماعز والأبقار في شمال فلسطين

ملخـص: تم اجراء هذه الدراسة لتحديد المسبب ومدى انتشار التهاب الضرع دون السريري في الأغنام ، الماعـز والأبقـار فـي شمال فلسطين. أظهرت هذه الدراسة أن تعداد الخلايا الجسمية (somatic cell count) فـي الحيوانات المصابة هو أعلى معنوياً من الحيوانات غير المصابة. زراعة البكتيريا أظهرت أن 52%، 72%، 59.1% مـن الماعز ، الأغنام والأبقار على التوالي مصابة بالتهاب الضرع دون السريري أغلـب الأصـابات 90.6% كانـت ناتجة عن الأصابة ببكتيريا موجبه لصبغة جرام ، حيث كانت بكتيريا المكورات العنقودية Staphylocci 68.2% هي السائدة كمسبب لالتهاب الضرع دون السريري ، كما تبين من نتائج إختبار إنزيم التخثر لبكتيريا Staphylocci أن 32.7% كانت موجبة التخثر ،35.6% كانت سالبة التخثر.هناك أنواع أخرى من البكتيريا المسببة لالتهاب الضرع تم عزلها وهي Micrococcus spp 18.3%، Proteus mirabilis 9.4% و Bacillus spp 4% . لهذا يعتبر التشخيص المبكر لالتهاب الضرع دون السريري مهم من ناحية إقتصادية وكذلك لتخفيف تطور التهاب الضرع السريري.

Abstract: This study was undertaken to determine aetiology and prevalence of subclinical mastitis in manually and mechanically milked animals in the north of Palestine. Milk samples from animals with bacterial infection of the mammary gland showed significantly higher somatic cell count (SCC) than did the corresponding milk from healthy animals, which (1,420±100 $X10^3$ cells/ml; vs. 330±35 $X10^3$ cells/ml; 1650±155 $X10^3$ cells/ml vs. 490±40 $X10^3$ cells/ml; 520±50 $X10^3$ cells/ml vs. 140±25 $X10^3$ cells/ml) for ewes, goats and cows, respectively. The prevalence of bacterial isolation of the milk from goats (n = 25), sheep (n = 40) and cows (n=220) from several major herds was determined. Culturing for bacteria revealed that 52 %, 72.5% and 59.1% of tested goats, sheep and cows had subclinical mastitis,

respectively. Most pathogens (90.6%) isolated from milk samples were Gram-positive bacteria. Staphylococci (68.3%) were the predominant cause of subclinical mastitis. Coagulase-negative staphylococci and coagulase-positive staphylococci accounted for 35.6% and 32.7% of the total bacteria isolated, respectively. Other mastitis pathogens isolated include Micrococcus spp (18.3%), *Proteous mirabilis* (9.4%) and Bacillus spp (4.0%). Early diagnosis of subclinical mastitis in dairy animals may be important in reducing production losses and enhancing prospects of recovery herds in order to avoid the development of clinical mastitis.

Keywords: subclinical mastitis/ Palestine/ microorganisims/ SCC

Introduction

Subclinical mastitis although it does not lead to visible changes in the milk or udder, it is more important economically than clinical mastitis due its higher prevalence, associated decrease in milk yield and altered potential quality and physicochemical properties of milk (Hamed *et al*., 1993; Dario *et al*., 1996; Urech *et al*., 1999). Despite subclinical mastitis occurs worldwide, it is considered as an important source of economic losses both in cattle and small ruminants in the Mediterranean area (Fthenakis 1994; Stefanakis *et al*., 1995; Seegers *et al*., 2003). The high diversity of pathogens responsible for subclinical mastitis has been reported and it makes an identification of the microorganisms in domestic ruminants significant, in order to establish specific and efficient management of dairy flocks to avoid the development of clinical mastitis (Lafi *et al*., 1998; Las Heras *et al*., 1999; Leitner *et al*., 2001; McDougall *et al*., 2002).

The somatic cell count (SCC) is an indicator of the intensity of the cellular immune defense and it represents a marker of the sanitary state of the udder. During the course of intramammary infection, leucocytes migrate from the blood towards the mammary gland leading to increase somatic cells in the milk. SCC represents a valuable tool for prevalence assessment and screening mastitis, a common accepted SCC values have not been established (Gonzalez-Rodriguez *et al*., 1995; Gonzalo *et al*., 1994; 2002).

In Palestine, most of the cattle and sheep farms are of the semi intensive type. Management of herds is the whole family activity especially females who deals with most of the activities from milking the animals to making milk products. The nutrition status of most livestock herds is above the average, where it meets the recommended standards by NRC. Most of herds are raised in hilly areas with hot dry summers and rainy cold winters.

In Palestine, the prevalence of subclinical mastitis has not been studied. The objectives of this study were to determine the prevalence of subclinical mastitis and to identify the pathogens that causing intramammary infection in both cows and small ruminants in several major herds in Northern Palestine, as this has not been investigated previously.

Materials and Methods

A total of 285 raw milk samples local goats (n=25), Awassi sheep (n=40) and Fresian cows (n=220) from several major herds in the north of Palestine were enrolled in this study. All goats and sheep in the present study which form a small sample were milked manually while all cows were mechanically milked. None of these animals were diagnosed with clinical mastitis and mammary glands without clinical abnormalities and giving apparently normal milk. Samples were collected into sterilized screw cap sample bottles between May and July of 2003. One milk sample (20-30 ml) was taken aseptically from each mammary gland after washing with warm water and cleaning the teats with cotton soaked in 70% alcohol and previous discard of the first three streams of milk. The samples were immediately taken in a container containing ice cubes to the laboratory for bacteriological analysis and somatic cell count.

Each milk sample (10µl) was surface plated on 5% sheep blood agar, MacConkey agar and nutrient agar. Samples were subsequently incubated at 37°C for 24-72h under aerobic conditions. Gram stain and culture characteristics (colony morphology, pigmentation, and hemolysis) were used for presumptive identification for all isolates. Further inoculations were done to confirm identification of the isolates biochemicaly. An intramammary infection was diagnosed when >500 CFU/ml of each of colony type was isolated (McDougall *et al*., 2002). Somatic cell counts were performed by a direct microscopic method.

Result

Milk from bacteriologically positive animals (infected) exhibits a significantly higher somatic cell count from both cows and small ruminants (ewes and goats) which no bacteria were isolated. Geometric range and mean of somatic cell counts from healthy and infected animals are represented in Table 1. The geometric mean of SCC from infected and healthy animals were (1,420±100 $X10^3$ cells/ml; vs. 330±35 $X10^3$ cells/ml;

1650±155 $X10^3$ cells/ml vs. 490±40 $X10^3$ cells/ml; 520±50 $X10^3$ cells/ml vs. 140±25 $X10^3$ cells/ml) for ewes, goats and cows, respectively.

The estimated prevalence of subclinical mastitis in the tested animals is shown in Table 2. The averages of subclinical mastitis detected in this study were 52, 72.5 and 59.1% with respect to goats, ewes and cows, respectively. The frequency of isolation of the bacterial species isolated and their distribution between animals is indicated in Table 2. Most pathogens (n=183) 90.6% isolated from milk samples were Gram-positive bacteria. Staphylococci (n=138) 68.3% were the most prevalent bacteria that can cause subclinical mastitis. Coagulase-positive staphylococci (*S.* aureus) and coagulase-negative staphylococci (*S. epidermidis* and *S. saprophyticus*) representing (n=66) 32.7% and (n=72) 35.6% of the total bacteria isolated (n=202), respectively. Other pathogens isolated include Micrococcus spp (n=37) 18.3%, *Proteous mirabilis* (n=19) 9.4% and Bacillus spp (n=8) 4%. In general, there was diversity in the species isolated from these herds. Dual infection was recorded only in 30 cows from different herds but not in sheep and goats, this may be due to that they form a small sample.

Discussion

In this study, the milk samples from animals with bacterial infection of the mammary gland showed significantly higher geometric SCC than did the corresponding milk from healthy animals. In ewes, the somatic cell count from those animals that classified as uninfected with bacteria was reported as ranging from 260 $X10^3$ - 1,850 $X10^3$ cell/ml, while in infected ewes was 1,199 $X10^3$ - 10,747 $X10^3$ cell/ml (Fthenakis, 1994; Mavrogenis *et al*., 1995; Burriel, 1997; Leitner *et al*., 2001; McDougall *et al*., 2002). The somatic cell counts in our study agree with the previously published data for ewes. The variation in SCC between these studies might be due to different factors such as type of breed, lactation number, lactation period, volume of milk produced, animal age, methodology and or pathogens producing infections (Gonzalez-Rodriguez *et al*., 1995; Mavrogenis *et al*., 1995; Gonzalo *et al*., 2002).

Uninfected goats are reported as having somatic cell count of 5 X 10^4 cells /ml to 1,850 X 10^3 cells/ml, While infected goat glands had 3 $X10^5$ - 15.1 X 10^6 cells/ml with variation among species of pathogens in the degree of somatic cell count elevation (Dulin *et al*., 1982; Lerondelle *et al*., 1992;

Ryan *et al*., 1993; Paape and Capuco, 1997; McDougall *et al*., 2002). Our results according to SCC agree with the previously published data for goats.

Milk from healthy cows exhibits a physiological basal cell count, which varies between 50 $X10^3$ - 394 $X10^3$ cells/ml of milk depending on the age of the cow, type of breed and milking fractions. Infected cows with subclinical mastitis had a somatic cell count higher than from uninfected and can reach up to a few million cells per milliliter, but it is usually more than 250,000 cells/ml (Reneau 1986; Smith 1995; Urech *et al*., 1999). In the present study, our result in agreement with data previously published concerning SCC in cows.

The prevalence of subclinical mastitis recorded in our study, was higher compared with that reported in other countries. The prevalence of subclinical mastitis in sheep reported for countries such as in Israel, Greece, England, Wales, Vermont (USA) and Spain has ranged from 12%-37% (Las Heras *et al*., 1999; Watson *et al*., 1990; Watkins *et al*., 1991; De la Cruz *et al*., 1994; Stefanakis *et al*., 1995; Leitner *et al*., 2001; McDougall *et al*., 2002). The prevalence of subclinical mastitis in goats and cows also reported for countries, in Vermont (USA) 27.3% (McDougall *et al*., 2002), Kenya 28.7% (Ndegwa *et al*., 2000), Ethiopia 60.8% and 38.2% (Dego and Tareke, 2003) and (Workineh et al., 2002). The prevalence of subclinical mastitis differs among countries. This might be due to the differences in animal breed, management conditions and methodological approach used.

In this study, bacteriological analysis of milk samples from animals with subclinical mastitis revealed that these animals infected by both minor pathogens (Coagulase-negative staphylococci, Micrococcus spp. and Bacillus spp) and major pathogens (*S.* aureus). Staphylococcus was the bacterial genus most frequently isolated from milk samples of these domestic ruminants. Coagulase-negative staphylococci are the most prevalent and widespread species isolated in milk samples from subclinical mastitis. This is in agreement with other authors (Lafi *et al*., 1998; Las Heras *et al*., 1999; Leitner *et al*., 2001). Coagulase-positive staphylococci (*S. aureus*) one of the most prevalent bacteria in subclinical mastitis in dairy animals and had a high significance in this study, as in other studies (Kudinha and Simango, 2002; Suarez *et al*., 2002). The high prevalence of animals with subclinical mastitis infected with *S. aureus* is a result of bad management due to virulent strains of *S. aureus* which might cause severe clinical mastitis lead towards

culling of the affected domestic ruminants. Micrococcus spp was the third bacterial group in importance according to the distribution among animals. Information about the widespread distribution of Micrococcus is very limited. The prevalence of Micrococcus spp found in this work is much higher than that reported in cows (Dego and Tareke, 2003). In view of the widespread distribution and prevalence rates found in this study, a higher clinical significance may be inferred for this group of bacteria as aetiological agents of subclinical mastitis in cows.

To our knowledge this is the first survey to estimate the prevalence of subclinical mastitis and the pathogen that causing this infection in cows and small rumunants in Palestine. It can be concluded that the prevalence of subclinical mastitis is too high in both cows and small ruminants and could develop into clinical cases in the absence of bacteriological testing and appropriate drug administration. Therefore, measures should be taken to control this disease. Although the number of the animals tested in this study was not too large especially in case of sheep and goats, however, it represents a sample in Northern Palestine, giving a picture of the general situation in this part of the country. Further studies are needed to find the mode of transmission, histopathological examination, and real effects on milk quality and a real relationship between SCC and pathogens.

Acknowledgements

Sincere thanks to Ra'fat M. Nakhash and Rania S. Quisi for their collaboration in sampling collection. The help and cooperation of the farmers who participated in this investigation is gratefully acknowledged.

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Table 1. Geometric somatic cell count (Range and Mean X 10^3cells/ml) for milk samples from ruminants of ewes, goats and cows as having no IMI or having IMI (i.e. >500 bacterial cell/ml of milk sample)

species	status	Number	Range X10^3	Mean X10^3±S.D
Ewes	No growth	11	300-420	330±35
Ewes	Infected	29	1,200-1,850	1,420±100
goats	No growth	12	330-550	490±40
goats	Infected	13	750-2,160	1650±155
cows	No growth	71	60-180	140±25
cows	Infected	139	400-700	520±50

Table 2. Frequency of different bacteria isolated from subclincial mastitis in dairy cows, goats and sheep from major several herds in the north of Palestine.

	Cows (n=220)	Goats (n=25)	Ewes (n=40)
S. aureus	n=43 (19.5%)	n=6 (24%)	n=17 (42.5%)
S. epidermidis	n=47 (21.1%)	n=7 (28%)	n=12 (30.0%)
S. saprophyticus	n= 6 (2.7%)	n=0 (0%)	n=0 (0%)
Micrococcus spp	n=37 (16.8%)	n=0 (0%)	n=0 (0%)
P. mirabilis	n=19 (8.6%)	n=0 (0%)	n=0 (0%)
Bacillus spp	n=8 (3.6%)	n=0 (0%)	n=0 (0%)
prevalence Of subclinical mastitis	n=130 (59.1%)*	n=13 (52%)	n=29 (72.5%)

*Thirty cows had dual infection from different herds.